Selective responses of hormones involved in carbohydrate and lipid metabolism and properties of erythrocyte membranes during the menstrual cycle in premenopausal women consuming moderate amounts of alcohol[1–3]

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ABSTRACT The effects of chronic consumption of moderate amounts of alcohol on hormones associated with lipid and carbohydrate metabolism, plasma concentrations of triacylglycerol and cholesterol, insulin receptors on erythrocyte membranes, and erythrocyte membrane fluidity were studied during three phases of the menstrual cycle in 37 premenopausal women. Subjects were given either 30 g ethanol or an equienergetic fruit juice for three menstrual cycles in a crossover design. Blood samples were analyzed during the luteal, midcycle, and follicular phases. Administration of alcohol induced a significant rise in plasma glucagon and cortisol uniformly across the entire menstrual cycle. A similar rise in plasma growth hormone was observed at midcycle during the period when subjects consumed alcohol. A marginal effect was observed on cholesterol and somatomedin C concentrations. Insulin binding to erythrocyte ghosts was not affected by either alcohol or menstrual-cycle phase. Erythrocyte membranes were more fluid during the follicular phase than during the luteal phase of the menstrual cycle when the women were consuming the alcohol. There were no perceptible interactions between alcohol and phases of the menstrual cycle for the indexes studied, except membrane fluidity. *Am J Clin Nutr* 1995;62:751–6.

KEY WORDS Premenopausal women, alcohol, hormones, insulin receptors, membrane fluidity

INTRODUCTION

Unlike excessive alcohol intake, moderate alcohol consumption has been shown to have beneficial effects on lipid metabolism. In normal volunteers moderate alcohol intake increases high-density-lipoprotein (HDL) cholesterol (1) and decreases the risk of coronary heart disease (2, 3) but increases the risk of breast cancer (4). Heavy consumption by alcoholic subjects and baboons results in elevation of triacylglycerol associated with very-low-density lipoproteins (5, 6). Chronic heavy alcohol consumption leads to modification of cell membrane cholesterol content and phospholipid fatty acyl composition as part of a homeostatic mechanism to offset the membrane-disordering effects of ethanol (7). Alcohol consumption also affects carbohydrate metabolism (8). The effects are species-dependent and are also dependent on the dosage, on the time of last food intake, and on the way the alcohol was administered—chronically or acutely (9, 10). Alcohol consumption has been associated with weight reduction (11, 12) as well as obesity (13). Alcohol increases resting energy expenditure and appears to interfere with energy utilization (14). It is possible that energy from alcohol may not be utilized as efficiently as that from fat and carbohydrate (15). Alcohol also affects the endocrine system in both normal and diabetic subjects. In normal subjects, earlier studies showed that a high dose of ethanol stimulates adrenal catecholamine secretion and urinary excretion (16, 17) but inhibits luteinizing hormone (LH) secretion (18). Alcohol also affects cortisol (19) and dehydroepiandrosterone sulfate (20).

Menstrual cycle per se also affects lipid, carbohydrate, and energy intakes and metabolism and the hormones involved in their metabolism. Food intake is greater during the luteal phase than during the midcycle and follicular phases in humans (21) and monkeys (22, 23). Martini et al (24) evaluated food intakes over four to six menstrual cycles and reported significantly higher energy, protein, carbohydrate, fat, vitamin D, riboflavin, potassium, phosphorus, and magnesium intakes in the midluteal phase than in the midfollicular phase. In rats (25, 26) and monkeys (22, 27), food intake is dependent on circulating concentrations of ovarian hormones. The menstrual cycle affects plasma concentrations of insulin and growth hormone (28). Although effects of menstrual cycle on insulin receptors have been reported (29), we observed no effect on insulin receptors (30) in a study of premenopausal women fed diets of different fat contents. Erythrocyte membrane fluidity was affected by the dietary fat content (31), but the effects were modulated by menstrual-cycle status. Insulin and growth

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hormone are both involved in carbohydrate and lipid metabolism. Significant differences in plasma cholesterol and triacylglycerol concentrations have been observed during different phases of the menstrual cycle (32–34).

Several studies have reported effects of alcohol on steroidal hormones in premenopausal women with liver damage (35–37) and in normal perimenopausal and postmenopausal women (19, 38). However, studies describing the effects of moderate alcohol intake in premenopausal women on plasma hormones involved in carbohydrate and lipid metabolism are lacking.

This study was conducted to determine how moderate alcohol consumption interacts with normal cyclic changes in ovarian hormones and gonadotropins to modulate plasma concentrations of hormones involved in carbohydrate and lipid metabolism and to assess effects on cell membranes in healthy premenopausal women. Reichman et al (39) have reported those aspects of the present study relating to the effect of moderate alcohol intake on steroidal hormones, which are implicated in breast cancer in premenopausal women.

METHODS

The study protocol was approved by the institutional review boards of the National Cancer Institute and Georgetown University School of Medicine. Premenopausal women aged 21–40 y were recruited from the Beltsville, MD, area for the study. The selected participants were nonsmokers and had not used hormonal methods of contraception in the past 12 mo. The subjects had no history of metabolic or chronic disease, had reported no menstrual irregularities, were not pregnant or lactating for $\geq$ 1 y, were not taking medication regularly, and had no unusual dietary pattern. The characteristics of the study population were as follows ($\bar{x} \pm$ SD): age, 30.4 $\pm$ 4.7 y; weight, 64.8 $\pm$ 12.5 kg; height, 163.2 $\pm$ 6.6 cm; quetelet's index, 24.4 $\pm$ 4.6 kg/m^2; and age at menarche, 12.6 $\pm$ 1.4 y. The average reported drinks per week was 1.7 $\pm$ 1.4. Other characteristics are described elsewhere (39). The subjects were paired based on age and relative weight and were randomly assigned to one of two groups. All subjects were fed diets containing 35% of energy from fat, a ratio of polyunsaturates to monounsaturates to saturates (P:M:S) of $\approx$0.6:1.0:1.0, and 2.0 g dietary fiber $\cdot$ MJ^{-1} $\cdot$ d^{-1}. All meals were prepared in the Human Study Facility of the Beltsville Human Nutrition Research Center (BHNRC). Breakfast and evening meals on weekdays were consumed in the BHNRC dining facility, and carryout meals were provided for weekday lunches and all weekend meals. A 14-d rotating menu cycle formulated from commonly available foods was used to ensure variety and acceptability. Subjects were initially assigned to an energy intake considered appropriate for weight maintenance and adjustments were made in 840-kJ increments when necessary to maintain weight.

The study was divided into two periods of three menstrual cycles each. In a random crossover design the subjects were given either 30 g ethanol/d (mixed with citrus fruit juice) or a soft drink with an energy value equal to that of the alcohol (plus plain citrus juice) for three menstrual cycles. The alcohol or equivalent soft drink was consumed at home after dinner and subjects were required to refrain from driving or performing other potentially hazardous activities. Thirty-seven women completed all phases of the study.

Morning fasting blood samples were collected on one of the days during the midfollicular (proliferative; days 5–7), midcycle (days 12–15), and midluteal (secretory; days 21–23) phases during the third menstrual cycle of each study period. The phases of the cycle were ascertained by measuring follicular stimulating hormone (FSH), LH, estradiol, and progesterone (39). Venous blood samples were collected into EDTA (1.4 g/L), Trasylol (100 kU/L; FBA Pharmaceuticals, New York), bestatin (88 μmol/L), and citrate (23 mmol/L). Plasma was portioned and stored at -70 °C until analyzed. Triacylglycerol was measured enzymatically by the method of Bucolo and David (40). Plasma cholesterol was measured as described by Allain et al (41).

All hormone analyses were performed by radioimmunoassay. Plasma glucagon was measured by radioimmunoassay with Unger's antibody 04A as previously described (42). Plasma insulin, cortisol, growth hormone, and somatomedin C were determined with kits from Immunonuclear Corp, Stillwater, MN (catalog # 0600, 9900, 0700, and 53065, respectively), and DHEA-S was determined with a kit from Radioassay Systems Laboratory Inc, Carson, CA (catalog # 1014).

For somatomedin C determination, 250 μL acidified plasma was applied to C_{18} Sep-Pak cartridges (Waters Associates, Milford, MA) activated with isopropyl alcohol, followed by methanol and 4% acetic acid. The cartridges were then washed with 4% acetic acid and the adsorbed somatomedin C was eluted with methanol. The eluate was dried in a Speed Vac (Savant Instruments, Farmingdale, NY) centrifuge. The lyophilized samples were reconstituted in assay buffer for analysis.

Insulin receptors and membrane fluidity were determined with erythrocyte ghosts prepared from blood samples drawn into tubes with anticoagulant but without Trasylol. After removal from plasma and platelets by differential centrifugation (1500 $\times$ g for 30 min at 4 ° C), the erythrocytes were dispersed in isotonic phosphate buffer (310 mOsmol, pH 7.4) and washed by repeated centrifugations (1000 $\times$ g for 20 min at 4 ° C). Erythrocyte ghosts were prepared by hypotonic lysis in 20 mOsmol phosphate buffer (pH 7.4) according to the procedure of Dodge et al (43). Ghosts were washed repeatedly in 20 mOsmol phosphate buffer to remove hemoglobin and other cytoplasmic components. Aliquots were removed for fluidity measurement, and the remainder of the ghost preparations was stored at -70 °C for assay of insulin binding.

Fluidity was assessed with freshly prepared membranes as a function of temperature between 5 and 35 °C by determining the anisotropy of fluorescence from the probe 1,6-diphenyl-1,3,5- hexatriene (DPH) according to the methods of Shinitzky and Barenholz (44). DPH (2 mmol/L in tetrahydrofuran) was diluted 1000-fold into the aqueous membrane suspension, which was then incubated with agitation at 35–37 °C for 2 h.

Steady state fluorescence polarization was measured with a spectrophotofluorometer (model 4800; SLM-Aminco, Rochester, NY) equipped with Glan-Thompson prism polarizers in the T-optical format. Excitation and emission wavelengths were 366 and 460 nm, respectively. Light-scattering errors were minimized by ensuring that the anisotropics were concentration-independent. DPH phase and modulation lifetimes were measured at 10 and 30 °C with oyster glycogen (Sigma, St Louis) as a scattering reference. Lifetimes were measured at two frequencies by setting the Debye-Sears ultrasonic

modulator at 18 and 30 MHz, and lifetimes were resolved by using Weber's (45) analysis.

Insulin binding was measured by incubating ghosts (100 μg protein/tube) in 0.5 mL Tris-HEPES buffer containing 40 mg bacitracin/L at either 10 °C for 4 h or at 30 °C for 2 h with 0.1 ng ^{125}I-labeled insulin (specific activity 81.4 TBq/mmol) and 0–100 mg native porcine insulin/L. ^{125}I-labeled insulin was purchased from New England Nuclear (Boston) and native porcine insulin was a gift from Eli Lilly and Co, Indianapolis. After the incubation, 0.2-mL aliquots were layered over 0.2 mL chilled Tris-HEPES buffer and centrifuged for 60 s at 7500 × *g* at room temperature (Microfuge II; Beckman Instruments, Inc, Fullerton, CA). The ghost pellet was washed once with 10% sucrose, and radioactivity was determined in a gamma counter (model A5550; Packard Instrument, Downers Grove, IL).

Statistical analysis

Data were analyzed statistically by analysis of variance (ANOVA) using the general linear models procedure to study the effect of alcohol and its interaction with menstrual-cycle phases (46). P values < 0.05 were considered statistically significant. When significant main effects of drink or menstrual cycle were observed, the data were further analyzed by Tukey's Studentized range test.

RESULTS

There was no significant effect of moderate alcohol intake or menstrual-cycle phase on plasma lipid concentrations in premenopausal women (**Table 1**). Similarly, no interactions were observed between the type of drink (alcohol compare with soft drinks) and the phases of the menstrual cycle for either triacylglycerol or cholesterol.

Effects of alcohol on plasma hormones involved in carbohydrate and lipid metabolism are shown in **Tables 2 and 3.** Moderate alcohol consumption tended to lower plasma insulin during the follicular and luteal phases but not during the midcycle phase. However, the overall effect was not statistically significant by ANOVA ($P < 0.07$). Alcohol consumption significantly decreased plasma somatomedin C concentrations whereas plasma concentrations of glucagon and cortisol were higher when women were consuming alcohol than when consuming the equienergetic beverage.

TABLE 1
Effect of moderate alcohol consumption on plasma triacylglycerol, and cholesterol during different phases of the menstrual cycle[1]

Drink and menstrual-cycle phase	Triacylglycerol	Cholesterol
	mmol/L	
Soft drink		
Follicular	1.03 ± 0.06	4.37 ± 0.18
Midcycle	0.99 ± 0.10	4.06 ± 0.25
Luteal	0.88 ± 0.09	3.90 ± 0.12
Alcohol		
Follicular	1.07 ± 0.09	4.30 ± 0.16
Midcycle	0.92 ± 0.06	4.05 ± 0.11
Luteal	0.89 ± 0.07	3.71 ± 0.20

[1] $\bar{x}$ ± SEM; $n = 37$. There were no significant differences by ANOVA.

TABLE 2
Effect of moderate alcohol consumption on plasma insulin, somatomedin C, and growth hormone during different phases of the menstrual cycle[1]

Drink and menstrual-cycle phase	Insulin	Somatomedin C[2]	Growth hormone[3]
	pmol/L	*nmol/L*	*μg/L*
Soft drink			
Follicular	78.4 ± 5.9	32.4 ± 1.2^{ab}	3.60 ± 1.06^{b}
Midcycle	82.5 ± 8.0	35.0 ± 1.7^{a}	3.66 ± 1.05^{b}
Luteal	88.4 ± 6.8	36.0 ± 0.9^{a}	1.76 ± 1.08^{c}
Alcohol			
Follicular	66.7 ± 6.7	29.9 ± 1.6^{b}	3.12 ± 1.00^{b}
Midcycle	85.0 ± 5.7	31.8 ± 1.0^{ab}	6.65 ± 1.42^{2}
Luteal	76.3 ± 4.9	33.9 ± 1.1^{ab}	3.13 ± 1.11^{b,2}

[1] $\bar{x}$ ± SEM; $n = 37$. Means with different superscript letters within the same column are significantly different, $P < 0.05$. (Tukey's Studentized range test).
[2] There was a significant overall treatment effect on somatomedin C concentrations, $P = 0.05$ (ANOVA).
[3] There was a significant overall effect of menstrual-cycle phase on growth hormone concentrations, $P = 0.034$ (ANOVA).

Although there was no significant effect of moderate alcohol on plasma growth hormone by ANOVA, Tukey's Studentized range test showed a significant effect of alcohol consumption on growth hormone during the midcycle and luteal phases. Growth hormone concentrations were significantly lower with both drinks during the luteal phase than during the midcycle phase. The concentration of plasma somatomedin C appeared to increase with progression of the menstrual cycle from menses to the secretory phase. However, the changes were not statistically significant by ANOVA ($P < 0.07$). Plasma glucagon concentrations were significantly lower during the luteal phase than during the follicular and midcycle phases according to Tukey's Studentized range test when women were consuming alcohol.

Neither menstrual cycle nor alcohol consumption had any significant effect on insulin binding to erythrocyte membranes when measured at 10 or 30 °C (**Table 4**). Alcohol had no

TABLE 3
Effect of moderate alcohol consumption on plasma glucagon and cortisol during different phases of the menstrual cycle[1]

Drink and menstrual-cycle phase	Glucagon	Cortisol
	pmol/L	*nmol/L*
Soft drink		
Follicular	30.8 ± 2.7^{ab}	456 ± 24^{b}
Midcycle	30.4 ± 1.7^{b}	456 ± 14^{b}
Luteal	26.2 ± 3.6^{bc}	415 ± 23^{c}
Alcohol		
Follicular	35.3 ± 2.6^{a}	508 ± 13^{a}
Midcycle	36.0 ± 2.9^{a}	477 ± 26^{a,b}
Luteal	30.8 ± 1.6^{b}	484 ± 24^{ab}

[1] $\bar{x}$ ± SEM; $n = 37$. Means with different superscript letters within the same column are significantly different, $P < 0.05$ (Tukey's Studentized range test). There was a significant overall treatment effect on glucagon ($P = 0.023$) and cortisol ($P = 0.014$) concentrations (ANOVA).

TABLE 4
Effect of moderate alcohol consumption on insulin binding to red cell ghosts during different phases of the menstrual cycle[1]

Drink and menstrual-cycle phase	Insulin binding at 10 °C	Insulin binding at 30 °C
	% specific binding/100 μg protein	
Soft drink		
Follicular	0.70 ± 0.05	0.93 ± 0.02
Midcycle	0.66 ± 0.07	0.92 ± 0.02
Luteal	0.72 ± 0.03	0.98 ± 0.04
Alcohol		
Follicular	0.66 ± 0.06	0.90 ± 0.10
Midcycle	0.68 ± 0.05	1.03 ± 0.03
Luteal	0.63 ± 0.09	0.94 ± 0.06

[1] $\bar{x}$ ± SEM; n = 37. There were no significant differences by ANOVA.

significant effect on membrane fluidity determined by DPH polarization measurement at 4, 10, and 37 °C, shown as the steady state anisotropy data in **Table 5.** Membranes were more fluid at 10 and 37 °C during the follicular phase than during the luteal phase only during the alcohol consumption period as shown by the decrease in DPH anisotropy. Neither alcohol consumption nor menstrual cycle had any significant effect on DPH fluorescence lifetimes (**Table 6**), thus validating the assessment of fluidity from the steady state anisotropies.

DISCUSSION

In this study we demonstrated that prolonged moderate alcohol consumption affects hormones that are involved in carbohydrate and lipid metabolism. Earlier studies on the effect of alcohol on hormones were carried out in alcoholic women who also had liver damage (35–37). In the current study women were not alcoholic and also consumed a controlled diet thereby allowing us to discern the specific effects of alcohol. Reichman et al (39) reported results of this study showing that alcohol induced increases in total and bioavailable estrogen concentrations. London et al (19) showed that moderate alcohol consumption affects adrenal steroids in older women (age 50–60 y) still having regular menstrual periods. The increased concentrations of steroidal hormones stimulated by alcohol in premenopausal women may be causally linked to increased incidence of breast cancer (39); however, this needs to be established. In female rats chronic alcohol feeding for 10 d has been reported to suppress the LH surge observed after the treatment with gonadotropin from pregnant mare serum (47). Chronic alcohol treatment in male rats also decreases plasma LH concentrations and in addition decreases testosterone concentrations and testosterone receptors in the pituitary (48). Other studies have reported increased (49), decreased (50), or unaltered (51) LH concentrations in rats chronically fed alcohol. These studies indicate a clear effect of alcohol on pituitary gonadotropins. It is therefore possible that some of the effect of alcohol observed during the midcycle period in this study may be in part due to changes in LH concentrations and needs to be further explored. It is important to note that FSH concentrations are also high during the midcycle phase. Whether chronic alcohol consumption affects FSH concentrations and whether FSH has any effect on hormones involved in carbohydrate and lipid metabolism is not clear and needs to be studied.

Earlier reports have shown that menstrual cycle per se affects lipid metabolism (32–34, 52) and hormones that are involved in lipid and carbohydrate metabolism (28). Higher total cholesterol and triacylglycerol and lower HDL-cholesterol concentrations were observed in the follicular phase than in the luteal phase (34). In the present study cholesterol concentrations were not significantly different between the three phases of the menstrual cycle. This was also true when women were consuming alcohol. It is interesting to note that the energy requirements of women are higher during the luteal phase of the menstrual cycle (53, 54). It is possible that differences in lipid and energy metabolism during different phases of the menstrual cycle are due to changes in hormones involved in their metabolism. Thus, insulin concentrations were higher as observed in a previous study (28) and growth hormone concentrations were lower as observed in the present study during the luteal phase than during the follicular phase when subjects were not consuming alcohol.

Although alcohol consumption did not affect triacylglycerol or cholesterol concentrations, it did increase the concentrations of glucagon and cortisol, the hormones involved in their metabolism. It is possible that other indexes controlled by these hormones may be affected by alcohol. The increase in plasma cortisol during alcohol consumption may be due to a generalized effect on adrenal function because other steroid concen-

TABLE 5
Effect of moderate alcohol consumption on red cell membrane fluidity during different phases of the menstrual cycle[1]

Drink and menstrual-cycle phase	DPH anisotropy at 4 °C	DPH anisotropy at 10 °C	DPH anisotropy at 37 °C
Soft drink			
Follicular	0.304 ± 0.002	0.289 ± 0.002	0.213 ± 0.002
Midcycle	0.302 ± 0.003	0.287 ± 0.003	0.213 ± 0.002
Luteal	0.306 ± 0.002	0.291 ± 0.002	0.215 ± 0.002
Alcohol			
Follicular	0.303 ± 0.002	0.288 ± 0.002	0.211 ± 0.002
Midcycle	0.305 ± 0.002	0.290 ± 0.002	0.213 ± 0.002
Luteal	0.309 ± 0.002	0.294 ± 0.002	0.217 ± 0.003

[1] $\bar{x}$ ± SEM; n = 37. There were no significant differences by ANOVA. DPH, 1,6-diphenyl-1,3,5-hexatriene.

TABLE 6
Effect of moderate alcohol consumption on DPH fluorescence lifetimes in red cell membranes during different phases of the menstrual cycle[1]

Drink and menstrual-cycle phase	τ-DPH at 10 °C	τ-DPH at 30 °C
	ns	
Soft drink		
Follicular	11.91 ± 1.18	11.51 ± 1.19
Midcycle	12.03 ± 0.92	10.25 ± 0.38
Luteal	13.11 ± 1.12	12.96 ± 1.21
Alcohol		
Follicular	11.08 ± 0.40	10.75 ± 0.40
Midcycle	10.97 ± 0.63	11.49 ± 1.01
Luteal	11.80 ± 0.76	12.69 ± 1.22

[1] $\bar{x}$ ± SEM; n = 37. There were no significant differences by ANOVA.

trations were also higher in these women when they were consuming alcohol (39). Clevidence et al (55) reported follicular-phase data from the present study showing that alcohol did not affect total plasma cholesterol but did increase HDL cholesterol and decrease low-density-lipoprotein (LDL) cholesterol.

Changes in ovarian hormones or gonadotropins did not affect erythrocyte insulin receptors in the current study or in a previous study (30). Similarly, moderate alcohol consumption had no effect on insulin binding. It is, however, possible that alcohol consumption may affect insulin receptors in well-recognized target organs such as muscle, adipose, and liver, especially in heavy drinkers who develop cirrhosis of the liver. Chronic alcohol administration to rats decreased insulin binding to rat hepatocytes, caused postreceptor abnormalities, and also altered glucose metabolism (56). In humans, in vitro incubation of erythrocytes from normal subjects with alcohol decreases insulin binding (57). These effects on insulin binding may be a function of in vitro modification of membrane fluidity, in accord with our reports relating insulin binding to fluidity in red cell ghosts (58, 59). Alcohols dissolve in cell membranes, and there is a body of evidence that alcohols or other anesthetics alter membrane lipid order without changing membrane chemical composition (7). Animal studies have shown that chronic ethanol ingestion increases membrane fluidity and that the organism compensates and maintains the physiologically necessary membrane order and fluidity through induced changes in membrane fatty acid composition and cholesterol content (7). In vitro studies (60–62) have shown that alcohols alter membrane enzyme activities and LDL-receptor binding, presumably through increases in membrane fluidity. The moderate alcohol intake in the present study was probably not sufficient to induce such drastic fluidity effects to result in modification of membrane fatty acids. The absence of an effect of the alcohol on the fluorescence lifetimes (Table 6) is important, because decreases in excited-state lifetimes could result in underestimation of membrane fluidity from steady state data (7, 63).

In summary, moderate alcohol consumption alters some but not all pancreatic and adrenal hormones, which are involved in carbohydrate and lipid metabolism. In addition, changes in ovarian steroids and gonadotropins that normally occur in different phases of the menstrual cycle can also affect the pancreatic and adrenal hormones. It is therefore important to take into account the state of the menstrual cycle whenever metabolic or endocrine indexes are studied in premenopausal women. Thus, prolonged alcohol consumption in premenopausal women is associated with mild alteration in pancreatic and adrenal function, leading to some alteration in carbohydrate and lipid metabolism.

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